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Abstract 509

Potent and Selective Inhibition of AXL Receptor Tyrosine Kinase for the Treatment of Cancer

Susan L. Paprcka, Akshata R. Udyavar, Subhasree Sridhar, Dillon H. Miles, Yu Chen, Christine Tu, Sean Cho, Corinne N. Foley, Rebecca Grange, Manmohan R. Leleti, Sharon Zhao, Lixia Jin, Stephen W. Young, Jay P. Powers , Matthew J. Walters

Arcus Biosciences, Inc.; 3928 Point Eden Way, Hayward, CA 94545 (USA)

OVERVIEW

- AXL receptor tyrosine kinase (AXL) is a transmembrane protein overexpressed in a variety of cancers and certain immune cells.
- AXL expression is associated with poor prognosis in a variety of cancers.
- AXL signaling has been implicated in creating an immunosuppressive tumor microenvironment (TME) through both tumor-intrinsic[1,2,3] and immunomodulatory mechanisms[4,5] promoting resistance to various therapies.[6,7,8,9]
- Arcus Biosciences is developing novel and selective AXL receptor tyrosine kinase inhibitors for the treatment of cancer.

AXL BIOLOGY & DOWNSTREAM EFFECTS

- Binding of Gas6 ligand to AXL receptor on the tumor cell surface induces AXL autophosphorylation.[10]
- AXL activation induces PI3K-AKT pathway signaling, resulting in upregulation of genes associated with EMT[11], DNA damage repair[12] and suppression of genes involved in immune cell engagement and activation[4] leading to therapeutic resistance and immune evasion.

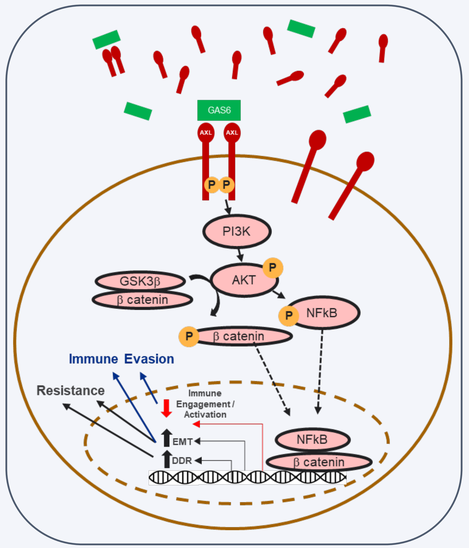

Figure 1. AXL Signaling Induced by Gas6. Binding of Gas6 to AXL leads to autophosphorylation of AXL, signaling through the PI3K-AKT pathway & induction of genes that drive therapeutic resistance & immune suppression.

RESULTS

AXL Expression is Associated with Poor Prognosis and Immunotherapy Resistance

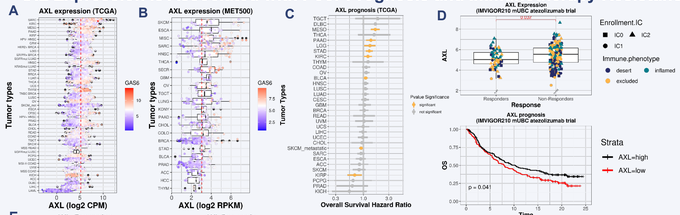

Figure 2. High AXL expression is associated with poor prognosis and resistance to immunotherapy. AXL is widely expressed and associated with high Gas6 expression across multiple cancers in both primary[13] (**A**) and metastatic[14] (**B**) settings. AXL is associated with poor prognosis in multiple cancer types in TCGA (**C**). High AXL expression is associated with poor prognosis and resistance to anti-PD(L)-1 therapies in metastatic bladder cancer (IMVIGOR210[15]) (**D**) and metastatic melanoma[16] (**E**).

AXL Expression is Associated with EMT and an Immunosuppressive TME

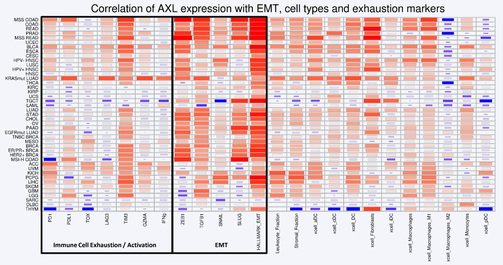

Figure 3. AXL expression is associated with EMT and an immunosuppressive TME. High AXL expression is correlated with the Hallmark EMT gene signature[17] as well as individual EMT genes, T-cell, fibroblast and myeloid infiltration and T-cell exhaustion.

AXL Expression is Highest in Dendritic Cells

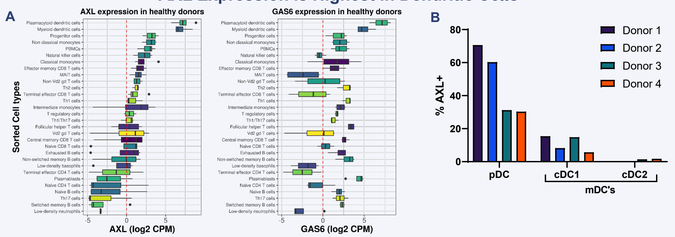

Figure 4. Dendritic cells have the highest expression of AXL and GAS6 among healthy immune cells. Other immune cells expressing lower or no AXL do express Gas6, which contributes to AXL signaling withing the TME. AXL RNA expression was evaluated in the Monaco et.al[18] dataset (**A**) and protein expression was evaluated in whole blood of healthy donors by flow cytometry (**B**). AXL protein was not expressed on T-cells, B-cells, NK cells, NK-T cells or monocytes in healthy donor whole blood (data not shown).

Characterization and Comparison of Novel Arcus and Benchmark AXL Inhibitors

Assay	Compound A	Compound B	Compound C	Bemcentinib[19]	Dubermatinib[19]	Sitravatinib[19]
hAXL HTRF IC_{50} (biochemical, nM)	2.8	1.8	0.95	5.2	77	0.98
mAXL HTRF IC_{50} (biochemical, nM)	0.95	0.90	0.62	2.7	--	--
hMERTK / hTYRO3 HTRF selectivity (biochemical, enzyme IC_{50} over AXL IC_{50})	130x / 39x	65x / 130x	104x / 92x	42x / 33x	14x / 9x	10x / 2x
hAXL NanoBRET™ K_D (cellular, nM)	13	9.1	5.8	135	1710	12
hAXL PathHunter® IC_{50} (cellular, nM)	36	15	24	340	1110	11
hAXL PathHunter® IC_{50} (cellular, nM) 100% serum	719	776	87	2270	2930	162

Table 1. Characterization and comparison of novel Arcus and benchmark AXL inhibitors. Kinase activity of AXL, MERTK and TYRO3 were tested using HTRF KinEASE – TK kit (CisBio) in the presence of 700 µM ATP. Inhibitor engagement with intracellularly expressed AXL kinase domain was detected using AXL NanoBRET™ TE intracellular kinase assay (Promega) with transiently transfected HEK293 cells. Compound activity in inhibiting SH2 domain translocation to phosphorylated AXL cytoplasmic domain upon AXL activation was tested using PathHunter® U2OS AXL functional assay (Eurofins DiscoverX). Cells were preincubated with inhibitors for 1hr followed by 3hrs Gas6 (1 µg/mL) induction. Assay was carried out in either a serum-free or 100% human serum medium.

AXL Inhibition Reverses Gas6-mediated Suppression of Soluble AXL

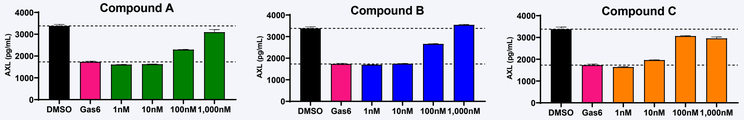

Figure 5. Novel AXL inhibitors Compounds A, B & C rescue soluble AXL levels that are reduced by Gas6. A549 human lung carcinoma cells were treated with 1 – 1,000 nM of Compound A, B or C for 1 h. 200 ng/mL hGas6 was added 1hr after addition of AXL inhibitors and cells were cultured for 72hrs. Supernatants were collected and soluble AXL levels were determined by ELISA. Similar results were observed in human PC-9, Panc-1 & MiaPaca2 cell lines (data not shown).

Compound A Demonstrates Target Engagement In Mice

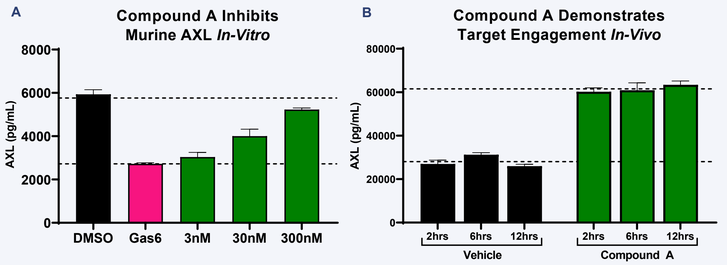

Figure 6. Compound A demonstrates target engagement in mouse. 4T1 murine breast cancer cells were treated with 1 – 300 nM of Compound A for 1hr. 500 ng/mL mGas6 was added 1hr after addition of AXL inhibitors and cells were cultured for 72hrs. Supernatants were collected and soluble AXL levels were determined by ELISA (**A**). Compound A was dosed at 100mg/kg BID in 4T1 tumor-bearing mice (n=7 per group) for 7 days. Plasma was collected at 2, 6 & 12hrs post-dosing on day 7. Soluble AXL levels (**B**) were evaluated. Compound A exposure exceeded IC_{90} levels adjusted for mouse potency in plasma.

RESULTS

AXL Inhibition Increases ICAM1 and MICA Expression Which Engage With & Activate T-cells and NK cells

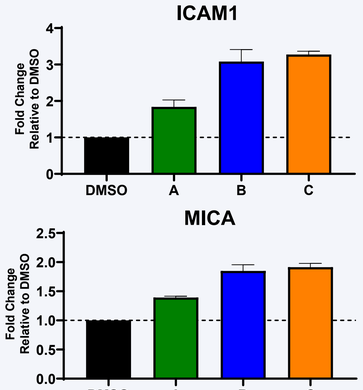

Figure 7. Novel AXL inhibitors, compounds A, B & C, upregulate gene expression of ICAM1 & MICA in human A549 lung cancer cell lines. Cell lines were treated with 1,000nM of Compound A, B or C and cells were cultured for 72hrs. RNA was isolated and gene expression was determined by qPCR. HPRT1 was used as a housekeeping gene.

SUMMARY

- AXL expression is associated with poor prognosis as well as tyrosine kinase inhibitor/immunotherapy resistance in several cancer types.
- AXL expression is highly associated with an immunosuppressive TME and is highly expressed in multiple cancer types.
- AXL is highly expressed by antigen presenting immune cells.
- Novel Arcus AXL inhibitors are potent and selective for AXL.
- Novel Arcus AXL inhibitors reverse Gas6-mediated suppression of soluble AXL in human and mouse AXL-positive cell lines.
- Compound A demonstrates target inhibition *in vivo*.
- Increased expression of ICAM and MICA, which meditate activation and engagement with T-cells and NK cells, is observed in tumor cells treated with novel Arcus AXL inhibitors.
- Arcus Biosciences is developing novel and selective AXL receptor tyrosine kinase inhibitor for the treatment of cancer.

REFERENCES

1) Gjerdrum et al. (2010) PNAS 107(3):1124-1129
2) Ying et al. (2019) Exp Ther Med 14:785-790
3) Tsukita et al. (2019) Mol Cancer 18:24
4) Terry et al. (2019) Cancer Immunol Res 7(11):1789-1820
5) Scutera et al. (2009) J Immunol 183:3004-3013
6) Wilson et al. (2014) Cancer Res 74(20):5878-5890z
7) Ludwig et al. (2018) Cancer Res 78(1):246-255
8) Brand et al. (2015) Cancer Res 74(18):5152-5164
9) Guo et al. (2017) Oncotarget 8(52):89761-89774
10) Linger, et al. (2008) Adv Cancer Res. 100:35-83
11) Wang, et al. (2016) Theranostics. 6(8):1205-1219
12) Balaji, et al. (2016) Mol Cancer Res. 15(1):45-58
13) Thorrson et al. (2018) Immunity 48:1-19
14) Robinson et al. (2017) Nature 548(7667):297-303
15) Mariathasan, et al (2018) Nature. 554:544-548
16) Hugo, et al. (2016) Cell. 24;165(1):35-44
17) Liberzon et al. (2015) Cell Syst. 23;1(6):417-425
18) Monaco et al. (2019) Cell Rep. 26:1627-1640
19) Data generated in-house. Compounds purchased from Synnovator (Bemcentinib), Advanced ChemBlocks (Dubermatinib), and Cayman Chemical (Sitravatinib)